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PAPER

Genetic factors as predictors of weight gain in young adult Dutch men and women

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OBJECTIVE: To investigate the association between DNA polymorphisms in several candidate genes for obesity and weight gain. Polymorphisms in these genes may contribute to weight gain through effects on energy intake, energy expenditure or adipogenesis.
DESIGN AND METHODS: From two large cohorts in the Netherlands (total 17 500 adult men and women), we compared 286 subjects aged 20–40 y who gained an average of 12.8 kg (range 5.5–47 kg) during a mean follow-up of 6.8 y with 296 subjects who remained relatively constant over the same period with respect to occurrence of several polymorphisms in candidate genes of obesity and some lifestyle factors. Subjects who were dieting, were high alcohol consumers, were pregnant, changed their smoking status recently, or those who suffered from serious illnesses were excluded. Polymorphisms were determined in the LEPR-gene (LEPR Lys109Arg, LEPR Gln223Arg, LEPR Lys656Asn), in the UCP1 gene (A–G mutation at position-3826 5′region), in the UCP2 gene (Ala55Val, 45 bp Ins/Del), in the PPARG2 gene (Pro12Ala) and in the ADRB2 gene (Gly16Arg and Gln27Glu).
RESULTS: With the exception of the Gly16Arg polymorphism in the ADRB2 gene in men ($P=0.04$) and women ($P=0.05$), and the Lys109Arg polymorphism in the LEPR gene in women, no statistically significant differences in the genotype and allele frequencies were observed between weight gainers and non-weight gainers. Weight gainers differed in some aspects of dietary habits and physical activity patterns: weight gainers consumed relatively more savory snacks and were less active during leisure time compared with non-weight gainers.
CONCLUSION: Only variations in the ADRB2 gene and LEPR gene, may contribute to susceptibility to weight gain. None of the other studied genetic markers were clearly associated with weight gain. Further research is necessary to establish the role of lifestyle factors, or interactions between genes or between genes and lifestyle factors on weight gain with age.
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Keywords: weight gain; epidemiology; genetics; candidate genes; obesity; leptin receptor; adrenergic receptor; uncoupling protein; peroxisome proliferator-activated receptor

Introduction

Obesity is increasingly common especially but not exclusively in affluent societies and is a risk factor for a number of chronic diseases.[1] Behavioral factors, such as physical activity level and dietary habits, are likely to be responsible for this increase in prevalence.[2,3] However, a large body of data suggests that genetic factors are also involved. These factors are thought to be associated with an increase in the predisposition to gain weight in the presence of adverse behavioral conditions.[4–8]

In recent years a large number of candidate genes have been suggested to be involved in the development of human obesity. However, for none of these candidate genes is the association with obesity strong and consistent.[8] This inconsistency might be explained by the different phenotypes used to assess obesity, eg body mass index (BMI), fat mass, or plasma leptin level. Another limitation of these candidate

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gene studies is that they have generally been based on small sample sizes or they were mostly performed in cross-sectional designs. Therefore, the genetic determinants for the development of obesity in the general population remain largely unidentified.

From a large general population, we have selected subjects whose weight remained relatively constant and subjects who gained weight over time. We described the characteristics and lifestyle factors of these two groups and we studied whether genetic variations are responsible for these differences in weight gain. We focused on nine common polymorphisms in five candidate genes which are potentially involved in the etiology of obesity: uncoupling protein genes (UCP1 and UCP2), which might play a role in energy expenditure and fat oxidation,[9] the leptin receptor (LEPR) gene which might be involved in the regulation of food intake and energy balance;[10,11] the peroxisome proliferator-activated receptor-γ2 (PPARG2) gene, which is thought to play a key role in adipogenesis,[12] and the adrenergic-β2 receptor (ADRB2) gene which is involved in the regulation of energy mobilization and utilization.[13]

Methods

Study population

Subjects were selected from participants in cardiovascular monitoring projects that have been carried out between 1987 and 1998 in two towns: Maastricht, a town in the south with about 100 000 inhabitants; and Doetinchem, a small town with about 40 000 inhabitants in the eastern part of The Netherlands. The initial aim of these projects was to monitor the major risk factors for cardiovascular disease. At baseline (in 1987–1991 and 1987–1997 for Doetinchem and Maastricht, respectively), men and women, aged 20–59, had height and weight measurements at the Municipal Health Services.[14] In Doetinchem, a second measurement occurred 6 years later in the period between 1993 and 1997, again at the Municipal Health Service. In Maastricht the follow-up weights were ascertained in 1998 by means of a self-administered questionnaire. Figures 1 and 2 show the time frame and the number of persons involved in the different projects in the two towns.

To exclude as much as possible the influence of potentially confounding factors for weight change, we excluded from these two cohorts all subjects at baseline and/or during the follow-up who reported to be on a diet, those consuming more than five glasses of alcoholic beverages per day, those with a history of chronic diseases, those who had recently changed their smoking habits (see Measurement section), those who were pregnant, and those with a follow-up of less than 4 years. Furthermore we excluded those of 40 years and above at baseline, because weight gain might be larger in the younger age groups.[15] In addition, 102 persons out of 17 743 were excluded for logistic reasons (eg no informed consent,

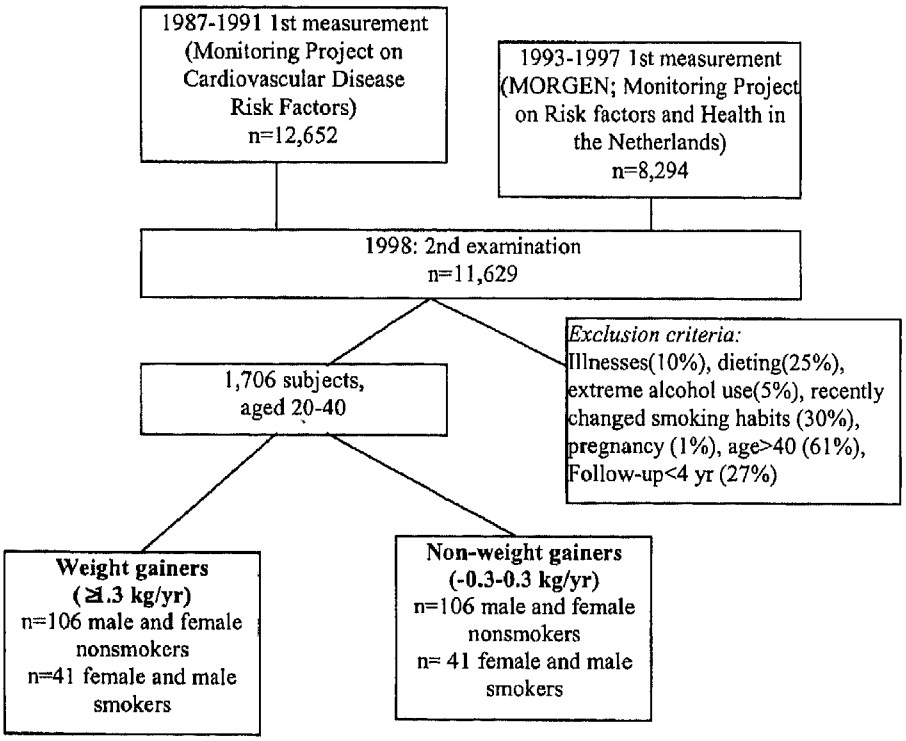

Figure 1 Selection of subjects from participants of cardiovascular monitoring projects in Maastricht.

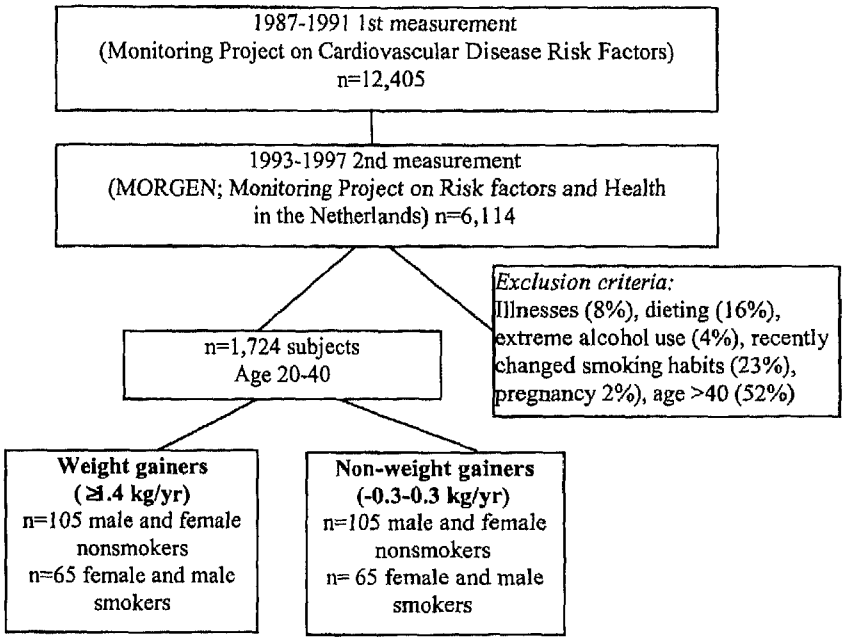

Figure 2 Selection of subjects from participants of cardiovascular monitoring projects in Doetinchem.

no blood sample, or no data on weight). From the remaining group (19% of the original sample), we selected the 'weight gainers' by taking the top decile of the distribution of average weight gain per year (≥ 1.4 and ≥ 1.3 kg/y for the cohorts of Doetinchem and Maastricht, respectively). We randomly selected an equal sized group of subjects (='non-weight gainers') whose weight remained relatively constant (range: -0.3 to $+0.3$ kg/y). The non-weight gainers were frequency matched for town, sex, age and smoking status with the weight gainers in a way that these two groups had not only the same marginal distributions but also the same joint distributions. All subjects had signed an informed consent to allow the use of stored blood samples for further scientific research. Finally, genomic DNA was successfully extracted from frozen blood samples for 296 weight gainers and 286 non-weight gainers.

Measurements

The examinations at baseline and the second measurement included physical examinations, eg anthropometric measurements, a self-administered questionnaire and blood sampling. However, for those in Maastricht, the second measurement included only a self-administered questionnaire.

Anthropometric measures. Weight at baseline was measured without shoes and wearing light indoor clothing. For all participants in Doetinchem weight was remeasured after 6 years in the same season as the initial measurement. For the participants from Maastricht, the second measurement was a self-reported weight. It is likely that subjects wore less clothing when measured at home compared with the measurement at the health center. To allow for the weight of clothing, we added 1.5 kg to the self-reported weight. This amount of 1.5 kg was based on some measurements performed by the investigators of the Municipal Health Center. BMI was calculated as weight divided by height squared. Weight gain was defined as the difference between the weight at baseline and the weight at the second examination. As the period of follow-up varied (6 y in Doetinchem and a range from 4.0 y to 11.3 y in Maastricht), we calculated the average weight gain per year.

Characteristics of the study population. The questionnaires at baseline and/or follow-up provided information about history of chronic diseases, alcohol consumption, cigarette smoking, pregnancy, and educational level. Subjects were considered to have a history of chronic diseases when they had a self-reported history of myocardial infarction, coronary artery bypass operation or another heart operation, stroke, cancer or diabetes mellitus. Smoking history was categorized into 'non-smokers' (those who never smoked or stopped smoking more than 5 y before the start of the study), 'smokers' (subjects who reported to be a current smoker at baseline as well as at the second measurement) and the remaining group (subjects who changed their smoking habits during the two measurements or stopped smoking less than 5 years before the start of the study). This last category was an exclusion category. The educational level,

a measure for socioeconomic status, was classified into three categories: low (primary school, lower vocational/general education), medium (intermediate/higher general education, intermediate vocational education), and high (higher vocational education, university).

Lifestyle factors. At baseline, subjects were asked in the questionnaire about their physical activity level during work and leisure time. The answer categories for these questions varied from 'mainly sitting' to 'heavy physical activity' and from 'little exercise' to 'heavy exercise', respectively (see Table 2). For physical activity during leisure time, the first category was classified as 'inactive', the other three as 'active'. Information on food consumption was collected by means of a short semi-quantitative food-frequency questionnaire. This questionnaire included foods with a high contribution to the nutrients of interest in cardiovascular disease epidemiology, eg type of edible fat, milk products, and meat consumption.[16] Information on physical activity and food consumption was available for 537 of the 582 subjects because these questions were only asked from 1987 to 1991.

Genotyping. Five candidate genes for energy intake, energy expenditure or adipogenesis which are of potential importance for weight gain, were selected. Genotyping was performed using PCR for UCP2 Ins/Del or PCR-restriction fragment length polymorphism (RFLP) analyses.

Two polymorphisms in the UCP2 gene were determined, a C to T nucleotide transition in exon 4 resulting in an Ala to Val substitution at codon 55,[17] and a 45 bp deletion/insertion in the 3′-untranscribed region of exon 8.[17] Furthermore we assayed an A to G transition at position-3826 5′region in the UCP1 gene[18] and a Prol2Ala missense mutation in the PPARG2 gene.[19] In the LEPR gene, we determined three polymorphisms: a lysine to arginine substitution at codon 109 (Lys109Arg), a glutamine to arginine substitution at codon 223 (Glu223Arg) and a lysine to asparagine substitution at codon 656 (Lys656Asn).[20] Finally, we investigated two polymorphisms in the ADRB2 gene, one at codon 16 substituting arginine for glycine (Gly16Arg) and one at codon 27 substituting glutamic acid for glutamine (Gln27Glu).[21] All genotyping methods are described in more detail elsewhere.[17–22]

Data analyses

Differences in characteristics, lifestyle factors, genotype and allele frequencies between weight gainers and non-weight gainers were analyzed with *t*-tests and chi-square tests. As weight at baseline was higher for the weight gainers compared to that of the non-weight gainers, we calculated weight-adjusted means and frequencies of the lifestyle and

Table 1 Characteristics of the study population (means (s.d.) or percentages)

	Men			*Women*		
	High weight gain (n = 134)	*Stable weight (n = 138)*	P-value	*High weight gain (n = 152)*	*Stable weight (n = 158)*	P-value
At baseline						
Age (y)	28.1 (5.9)	28.9 (5.6)	[a]	29.9 (6.0)	29.9 (5.9)	[a]
Smoking status (%)						
Nonsmokers	64.9	64.5	[a]	67.1	67.1	
Smokers	35.1	35.5		32.9	32.9	
Town (%)						
Doetinchem	53.7	49.3	[a]	63.2	59.5	[a]
Maastricht	46.3	50.7		36.8	40.5	
Weight (kg)	79.4 (10.8)	76.3 (10.0)	0.016	66.8 (11.9)	63.6 (8.0)	0.006
Height (cm)	1.82 (0.07)	1.80 (0.07)	0.020	1.68 (0.07)	1.67 (0.06)	0.27
BMI (%)						
$< 18.5\ kg/m^2$	0.0	2.2		3.3	3.2	
$\geq 18.5 - < 25.0\ kg/m^2$	68.7	65.2		65.8	76.0	
$\geq 25.0 - < 30.0\ kg/m^2$	26.9	30.4		23.9	19.0	
$\geq 30.0\ km/m^2$	4.5	2.2	0.13	7.3	1.9	0.068
BMI (kg/m^2)	24.1 (3.0)	23.7 (2.8)	0.45	23.8 (3.9)	22.9 (2.8)	0.02
Socioeconomic status (%)						
Low	47.8	42.0		57.2	48.1	
Medium	41.0	37.7		31.6	31.0	
High	11.2	20.3	0.12	11.2	20.9	0.056
At end of follow-up						
Follow-up time (y)	6.9 (1.7)	7.2 (1.9)	0.16	6.9 (1.5)	6.8 (1.6)	0.42
Weight (kg)	92.0 (11.5)	76.8 (10.0)	[b]	79.7 (13.3)	64.2 (8.0)	[b]
Weight gain (kg/y)	1.86 (0.53)	0.06 (0.17)	[b]	1.95 (0.62)	0.07 (0.16)	[b]
BMI (kg/m^2)	27.9 (3.3)	23.8 (2.8)	[b]	28.4 (4.6)	23.1 (2.7)	[b]

[a] *P*-values not given, one of the matching variables.
[b] *P*-values not given, criteria for group selection.

Table 2 Dietary habits and physical inactivity in weight gainers and those with stable weight, adjusted for baseline weight (means or percentages)

	Men			*Women*		
At baseline	*High weight gain (n = 123)*	*Stable weight (n = 127)*	*P-value*	*High weight gain (n = 142)*	*Stable weight (n = 147)*	*P-value*
Supplements (% users)	30.2	43.2	0.03	48.7	48.2	0.93
Sweeteners (% users)	7.1	1.0	0.02	14.3	11.5	0.48
Milk products (mean % of total milk consumptions)						
Skimmed milk (mean %)	25.5[a]	27.2	0.63	40.7[b]	36.8	0.33
Semi-skimmed milk (mean %)	39.8	32.0	0.06	31.8[b]	34.2	0.50
Full fat milk (mean %)	34.7[a]	40.8	0.20	27.5[b]	28.9	0.73
Fruit (%)						
$< \frac{1}{2}$ times a day	30.4	22.4		22.9	17.5	
$\geq \frac{1}{2}$ times a week– < 1 times a day	23.1	25.7		26.6	20.1	
≥ 1 times a day– < 2 times a day	38.6	43.7		33.9	50.2	
> 2 times a day	7.8	8.2	0.55	16.5	12.1	0.05
Vegetables (%)						
$< \frac{1}{2}$ times a day	12.7	16.1		14.2	9.5	
$\geq \frac{1}{2}$ times a week– < 1 times a day	47.8	36.4		34.2	38.2	
≥ 1 times a day– < 2 times a day	37.2	36.5		44.9	45.0	
> 2 times a day	2.3	11.1	0.02	6.7	6.5	0.65
Sweet sandwich filling (mean % of total number of fillings)	23.8	22.3	0.59	24.6[c]	29.6	0.05
Type of bread[d]						
White bread (% consumers)	27.9	20.3	0.17	16.5	13.5	0.46
Wheat bread (% consumers)	35.0	44.0	0.15	46.2	41.0	0.38
Wholemeal bread (% consumers)	41.7	43.0	0.84	42.5	50.8	0.16
Edible fats on bread[d]						
Low-fat margarine (% consumers)	46.4	51.9	0.39	51.6[e]	51.1	0.94
Margarine (% consumers)	44.0	36.9	0.26	30.5[e]	31.5	0.86
Butter (% consumers)	13.9	18.8	0.30	14.8[e]	17.5	0.54
No fat on bread (% consumers)	12.3	8.6	0.35	16.3[e]	16.8	0.90
Edible fats for dinner[d]						
Cooking fat[f] or butter (% consumers)	30.2[g]	23.7	0.26	29.7[h]	32.0	0.68
Margarine (% consumers)	64.4[g]	61.8	0.67	60.9[h]	61.9	0.87
Oil or linoleic acid-enriched margarine (% consumers)	34.8[g]	43.8	0.15	36.9[h]	38.9	0.74
Fish consumption (%)						
Never	8.0	17.5		13.1	13.4	
< 1 times a month	31.1	21.0		29.0	24.7	
≥ 1 times a month– < 1 times a week	37.1	40.0		28.4	30.9	
≥ 1 times a week	23.8	21.9	0.07	29.5	31.1	0.87
Savory snacks (%)						
< 3.5 times a week	21.3	27.3		20.9[b]	32.3[i]	
≥ 3.5 times a week– < 1 times a day	37.0	48.4		46.6[b]	45.3[i]	
≥ 1 times a day	41.6	24.2	0.01	32.5[b]	22.3[i]	0.04
Cookies or chocolate (%)						
< 3.5 times a week	53.0	52.6		38.6	39.4	
≥ 3.5 times a week– < 1 times a day	24.0	29.5		29.0	27.5	
≥ 1 times a day	22.9	18.0	0.49	32.4	33.1	0.96
Alcohol consumption (% abstainers)	13.0	4.8	0.02	24.7	20.7	0.43
Physical activity during working time (%)						
Mainly sitting	22.8	25.9		12.4	16.9	
Sitting/standing and sometimes walking	32.4	43.4		34.0	32.3	
Walking	32.9	19.3		37.2	38.0	
Physically active	6.1	4.3		5.8	1.2	
Not applicable	5.7	7.1	0.12	10.6	11.7	0.24
Physical activity during leisure time (%)						
Inactive[j]	32.1	24.0		44.3	31.8	
Active[k]	67.9	76.0	0.16	55.7	68.7	0.03

[a] $n = 121$ because two persons are not consuming milk products.
[b] $n = 141$.
[c] $n = 138$.
[d] These categories were not exclusive.
[e] $n = 139$
[f] Contains 97% fat, suitable for frying and baking.
[g] $n = 122$.
[h] $n = 140$.
[i] $n = 144$.
[j] Inactive = Little exercise.
[k] Active = Exercise for at least 4 hours a week/Regular exercise/Regular strenuous exercise.

genetic factors on the basis of analysis of covariance and logistic regression analyses. Subsequently, chi-square tests were done with the adjusted frequencies of the categorical variables.

Furthermore, we performed similar analyses in which the matching factors were also included in the model. To investigate the impact of more polymorphisms simultaneously on weight gain, we performed logistic regression analyses in which weight at baseline, two polymorphisms and its interaction term are included. To increase the power of these last analyses, the heterozygotes at each polymorphism were collapsed with the smallest group of homozygotes. For example, subjects with the Pro12/Ala12 and with the Ala12/Ala12 genotype in the PPARG gene were collapsed and compared with Pro12/Pro12. Genotype distributions of the non-weight gainers[23] were tested for Hardy–Weinberg equilibrium by chi-square analyses. All analyses were performed separately in men and women using the statistical package SAS (version 6.11) and a P-value of 0.05 was considered as statistically significant.

Results

The characteristics of the study population are shown in Table 1. Subjects were on average 29.2 (s.d. ± 5.9) y old and had a BMI at baseline of 23.6 kg/m^2 (s.d. ± 3.2). Subjects with high weight gain increased on average 12.8 kg (range 5.5–47 kg) during a mean follow-up of 6.8 y, while the non-weight gainers gained on average 0.5 kg (range −2.6–3.1 kg). At baseline, male weight gainers did not differ in socioeconomic status, from the non-weight gainers. Male weight gainers already had a higher body weight at baseline compared with the non-weight gainers. However, they did not have a higher mean BMI, as they were also 2 cm taller compared with the non-weight gainers.

Among women, the socioeconomic status, based on the educational level, was slightly lower for the weight gainers compared with the weight keepers ($P=0.056$). Furthermore, female weight gainers had a higher body weight, a similar height, and as a consequence a higher BMI at baseline compared with the female non-weight gainers. This difference in weight could not be explained by the differences in socioeconomic status (data not shown). The male and female weight gainers and non-weight gainers did not differ in mean blood pressure or mean plasma cholesterol concentration (data not shown).

As the unadjusted and the adjusted associations between weight gain and the lifestyle or genetic factors were mostly similar, only the weight-adjusted results are shown. Table 2 shows the weight-adjusted dietary factors and physical activity of the weight gainers and non-weight gainers. The male weight gainers used fewer vitamin and mineral supplements, but more sweeteners compared with the male non-weight gainers. Furthermore, the male weight gainers consumed slightly less full-fat milk products, but more semi-skimmed milk products compared with male non-weight gainers. No statistically significant differences were observed in fruit consumption, type of bread, sandwich filling, and type of edible fat used on bread or for dinner between these two groups. Compared to men with stable weight vegetables were less often consumed by male weight gainers. Male weight gainers tended to eat more frequently fish, beef or chicken (data not shown). Relatively more weight gainers were alcohol abstainers, while weight gainers consumed same type of alcoholic beverages (data not shown) compared to non-weight gainers. Finally, the male weight gainers consumed significantly more savory snacks compared with the non-weight gainers.

Among women, no statistically significant differences were observed in supplement use, in consumption of milk products, type of bread, type of edible fat, vegetables, meat (data not shown), or in alcohol consumption between the weight gainers and weight keepers. Fruit was less often consumed by the female weight gainers and their sandwiches were less often filled with sweet products compared with those of female non-weight gainers. Finally, also among women an association was observed between frequent use of savory snacks and weight gain. There were no statistically significant differences in physical activity during working time or during leisure time between the two male groups ($P=0.12$ and $P=0.16$, respectively). Among women, no significant differences were found in physical activity level during working time. However, during leisure time, non-weight gainers exercised more often compared with those who gained most in weight ($P=0.03$).

Table 3 shows the weight-adjusted genotype frequencies for all studied DNA-polymorphisms of the weight gainers and the non-weight gainers. No consistent associations were observed. The only statistically significant association with weight gain was found among men with the Gly16Arg polymorphism in the ADRB2-gene: weight gainers were more often homozygous, either Gly/Gly or Arg/Arg, and less often heterozygous for this polymorphism ($P=0.04$). This association was not observed among women, in fact the percentage of homozygotes for Gly/Gly was higher in the group of weight gainers compared with the non-weight gainers (not shown in table; $P=0.05$). Furthermore some (non-significant) trends were found among women between polymorphisms of the LEPR-gene and weight gain; weight gainers tended more often to be carriers of the Lys allele at codon 109 (allele frequencies Lys109 74% *vs* 68%; $P=0.09$); and the Arg/Arg genotype at codon 223 was slightly underrepresented in weight gainers (16% *vs* 25%; $P=0.07$). All genotype distributions were found to be in Hardy–Weinberg equilibrium with the exception of the distribution of ADRB2-Gly16Arg in the male non-weight gainers ($P=0.03$).

We also investigated the combined effect of two polymorphisms on weight gain. From all combinations, only a few (see Table 4) showed a statistically significant interaction term. For men, a significant interaction term was observed between the A(-3826)G polymorphism in the UCP-1 gene and the Lys656Asn polymorphism in the LEPR gene, as well

Table 3a Genotype frequencies for male weight gainers and those with stable weight, adjusted for weight at baseline (percentages)

	Men			
	High weight gain (n = 134)	*Stable weight (n = 138)*	*P-value*	OR[a] (95% CI[b])
UCP1 A (−3826)G (%)				
A/A	55.3	52.1		1 (ref)
A/G	38.1	38.4		0.93 (0.61–1.4)
G/G	6.6	9.5	0.66	0.65 (0.30–1.4)
UCP2 Ins/Del (%)				
Del/Del	55.3	52.1		1 (ref)
Ins/Del	38.1	38.4		1.14 (0.74–1.7)
Ins/Ins	6.6	9.5	0.66	0.94 (0.42–2.1)
UCP2 Ala55Val (%)				
Ala/Ala	45.5	48.5		1 (ref)
Ala/Val	39.2	38.1		1.10 (0.71–1.7)
Val/Val	15.3	13.4	0.85	1.22 (0.66–2.3)
LEPR Lys109Arg (%)				
Lys/Lys	56.4	53.9		1 (ref)
Lys/Arg	37.6	37.4		0.96 (0.63–1.5)
Arg/Arg	6.0	8.7	0.69	0.66 (0.29–1.5)
LEPR Gln223Arg (%)				
Gln/Gln	33.9	32.3		1 (ref)
Gln/Arg	46.4	48.5		0.91 (0.58–1.4)
Arg/Arg	19.7	19.2	0.94	0.98 (0.55–1.5)
LEPR Lys656Asn (%)				
Lys/Lys	60.3	66.9		1 (ref)
Lys/Asn	37.4	27.5		1.50 (0.97–2.3)
Asn/Asn	2.4	5.7	0.11	0.47 (0.15–1.5)
PPARG2 Pro12Ala (%)				
Pro/Pro	75.4	79.7		1 (ref)
Pro/Ala	23.1	20.3		0.85 (0.54–1.3)
Ala/Ala	1.5	0	0.29	1.05 (0.10–10.9)
ADRB2 Gly16Arg (%)				
Gly/Gly	43.9	35.6		1 (ref)
Gly/Arg	39.9	54.7		0.59 (0.38–0.92)
Arg/Arg	16.1	9.7	0.04	1.36 (0.70–2.6)
ADRB2 Gln27Glu (%)				
Gln/Gln	30.7	26.7		1 (ref)
Gln/Glu	53.0	55.8		0.83 (0.52–1.3)
Glu/Glu	16.3	17.5	0.77	0.81 (0.44–1.5)

[a]*Odds rations.* [b]*Confidence interval.*

as between the Lys109Arg polymophism in the LEPR gene and the UCP2 Ins/Del polymorphism. The risk of high weight gain was the highest for men with the A/A genotype and one or two Asn656 alleles (OR 2.4, 95% CI 1.1–4.9), and for men with one or two Ins alleles and also one or two Arg109 alleles (OR 1.14, 0.54–2.4) Furthermore, significant effects were observed among women for the combination of Gly16Arg polymorphism in the ADRB2 gene and the UCP2 Ins/Del polymorphism, and for the combination of the same polymorphism in the ADRB2 gene and the PPARG2 Pro12Ala polymorphism: the risk on high weight gain was the largest for women with the Del/Del and Arg16/+ genotypes (OR 2.7, 1.3–5.4), and for the female carriers of the Ala 12 and also the Arg16 alleles (OR 1.6, 0.8–3.4).

Discussion

We observed that one polymorphism in the ADRB2 gene and one in the LEPR gene, but none in the other candidate genes was significantly associated with weight gain. In addition, some aspects of dietary habits and physical activity patterns were associated with weight gain.

A number of issues need to be addressed before the results can be interpreted. First, the issue of potential selection bias. The participation rate in the original cohort was about 50%.[14] It is unlikely, however, that among the weight gainers and those who kept stable weight there was selective participation by genotype. Furthermore, we excluded subjects on the basis of some baseline measurements and measurements at the end of the follow-up. However, as not all measurements were for 100% representative of the situation during the follow-up, it is still possible that some of the differences between the weight gainers and weight stable group can be attributed by differences in the selection criteria.

Among the male non-weight gainers we observed a slight departure from a Hardy–Weinberg Equilibrium (HWE) for the Gly16Arg polymorphism in the ADRB2 gene. Prudence is called for drawing conclusions from this, since it is still possible that the general population is in HWE; Schaid *et al* mentioned that, when a true association exists, the expected

Table 3b Genotype frequencies for female weight gainers and those with stable weight, adjusted for weight at baseline (percentages)

	Women			
	High weight gain (n = 152)	*Stable weight (n = 158)*	P-value	OR[a] (95% CI[b])
UCP1 A (−3826)G (%)				
A/A	62.3	58.4		1 (ref)
A/G	30.3	35.4		0.80 (0.53–1.2)
G/G	7.4	6.1	0.61	1.13 (0.53–2.4)
UCP2 Ins/Del (%)				
Del/Del	62.3	58.4		1 (ref)
Ins/Del	30.3	35.4		0.99 (0.67–1.5)
Ins/Ins	7.4	6.1	0.61	1.00 (0.48–2.1)
UCP2 Ala55Val (%)				
Ala/Ala	37.7	37.2		1 (ref)
Ala/Val	43.6	42.3		1.02 (0.67–1.6)
Val/Val	18.8	20.6	0.92	0.90 (0.53–1.5)
LEPR Lys109Arg (%)				
Lys/Lys	56.0	48.6		1 (ref)
Lys/Arg	36.5	38.9		0.82 (0.54–1.2)
Arg/Arg	7.4	12.5	0.23	0.52 (0.26–1.0)
LEPR Gln223Arg (%)				
Gln/Gln	31.4	29.2		1 (ref)
Gln/Arg	52.3	45.9		1.06 (0.68–1.6)
Arg/Arg	16.3	24.9	0.17	0.61 (0.35–1.1)
LEPR Lys656Asn (%)				
Lys/Lys	69.1	65.1		1 (ref)
Lys/Asn	27.4	31.9		0.81 (0.53–1.2)
Asn/Asn	3.5	3.0	0.68	1.17 (0.38–3.3)
PPARG2 Pro12Ala (%)				
Pro/Pro	76.7	73.7		1 (ref)
Pro/Ala	22.6	25.7		1.20 (0.73–2.0)
Ala/Ala	0.7	0.6	0.81	-
ADRB2 Gly16Arg (%)				
Gly/Gly	34.7	45.7		1 (ref)
Gly/Arg	53.3	44.5		1.58 (1.1–2.4)
Arg/Arg	12.2	9.8	0.14	1.63 (0.85–3.1)
ADRB2 Gln27Glu (%)				
Gln/Gln	34.4	31.5		1 (ref)
Gln/Glu	45.7	49.1		0.86 (0.56–1.3)
Glu/Glu	19.9	19.4	0.82	0.94 (0.55–1.6)

[a]Odds ratios. [b]Confidence interval.

genotype proportions among the diseased can deviate from HWE even if the general population is in HWE.[23] In our study this can be argued for the weight gainers as well as the non-weight gainers. Therefore, we do not assume that the departure accounts for the association found between the polymorphism in the ADRB2 gene and weight gain.

An advantage of our study was our prospective design to study simultaneous the association between lifestyle factors or genetic variation and weight. As lifestyle factors might affect weight gain, and a higher weight could in turn influence someone's lifestyle, cross-sectional and longitudinal studies on the etiology of obesity can generate completely opposite conclusions.[24] Our findings could still be affected by misclassification problems. For the subjects from Maastricht, the weights at the end of the follow-up were self-reported. As errors in self-reported weight increase with the magnitude of overweight, this misclassification is probably not randomly distributed.[25] However, we assumed that the ranking among weight gainers and those who remained weight stable was not affected by self-report. Admittedly, information on dietary food frequency and physical activity was relatively crude and does not allow careful evaluation of their role as predictor of weight gain. Instead, this information should be interpreted for the purpose of description rather than hypothesis testing. One advantage compared to cross-sectional studies on obesity and lifestyle factors is that we collected information on these variables when the subjects were still lean and the bids of misreporting may have been excluded.

Finally, some factors, such as weight at baseline or the matching factors could have confounded our results. Fortunately, these biases were of minor importance, as similar results were found with and without adjustment for baseline weight or the matching factors (data not shown).

The published evidence for associations between candidate genes and obesity-related phenotypes is conflicting. For instance, associations were found between the Arg16 variant of the ADRB2 gene with BMI, waist and hip circumferences among French men,[26] and with BMI among Japanese women,[27] but not among French women and Japanese

Table 4 The significant combinations of two polymorphisms on the risk of weight gain (odds ratios)

	Polymorphism 2				
Polymorphism 1	*n/n*[a]	*OR*[b] *(95% CI)*	*n/n*	*OR (95% CI)*	P-Interaction
Men	LEPR Lys656Asn				
UCP1 A(−3826)G	Lys/Lys		Lys/Asn or Asn/Asn		
A/A	44/54	1 (ref.)	31/17	2.37 (1.1–4.9)	
A/G or G/G	37/38	1.30 (0.70–2.4)	22/29	0.98 (0.49–2.0)	0.030
Men	LEPR Lys109Arg				
UCP2 Ins/Del	Lys/Lys		Lys/Arg or Arg/Arg		
Del/Del	43/32	1 (ref.)	29/44	0.53 (0.27–1.0)	
Ins/Del or Ins/Ins	33/42	0.64 (0.33–1.2)	29/20	1.14 (0.55–2.4)	0.016
Women	ADRB2 Gly16Arg				
UCP2 Ins/Del	Gly/Gly		Gly/Arg or Arg/Arg		
Del/Del	21/39	1 (ref.)	50/35	2.69 (1.3–5.4)	
Ins/Del or Ins/Ins	32/33	1.77 (0.85–3.7)	49/51	1.78 (0.91–3.5)	0.040
Women	ADRB2 Gly16Arg				
PPARG2 Pro12Ala	Gly/Gly		Gly/Arg or Arg/Arg		
Pro/Pro	44/49	1 (ref.)	73/67	1.19 (0.70–2.0)	
Pro/Ala or Ala/Ala	9/23	0.41 (0.17–0.99)	26/9	1.64 (0.79–3.4)	0.035

[a]Number of persons with high weight gain/number of persons with stable weight.
[b]Odds ratios.

men. Furthermore, positive and negative associations were found between the Glu27 variant of the ADRB2 gene with BMI, fat mass or fat cell volume in French, Swedish and Japanese subjects.[21,38,39] Also, associations were found between polymorphisms in the UCP2 gene and weight gain, obesity or metabolic rates,[17,30,31] between variants in the UCP1 gene and weight gain, weight, or BMI loss,[32–34] between polymorphisms in the LEPR gene and percentage body fat or BMI,[35–37] and between polymorphisms in the PPARG2 gene and BMI, gain in BMI or leptin levels.[38–42] However, studies showing no associations for these polymorphisms have also been published.[22,37,42–51] In the present study, we could not find associations between all of these polymorphisms with weight gain in men and women. These essentially negative results might be explained by several factors. First, it is important to recognize that we have used only one among several potentially useful phenotypes. Indeed, our design is a comparison between high weight gainers and non-weight gainers. It would be useful also to investigate the effect of the genetic polymorphisms on the distribution of weight gain adjusted for baseline body weight, separately in men and women. Moreover, since in the regulation of body weight food-seeking behavior, energy expenditure and complex interactions among these traits are likely to be involved, not for all obesity phenotypes are similarly associated with these polymorphisms. However, even the few studies on the association between genetic factors and weight gain showed inconsistent results. One study also reported a lack of association between the UCP2 Ins/Del and weight gain in Danish Caucasians[47] but Clement *et al* reported an association between the UCP1 A(-3826)G polymorphism and weight gain.[32] However, the latter was performed on morbidly obese patients, while we investigated the impact of genetic factors on weight gain in a general population.

Another reason for the apparent discrepancies in results may have to do with lack of statistical power. Although we have selected our study population in order to get the largest contrast, the number of subjects might still be too small to be able to detect a significant association, especially for a relatively rare polymorphism such as the Pro12Ala mutation in the PPARG2 gene. Therefore, it would be useful to investigate these associations in a larger population. Thirdly, differences in ethnic background could account for some of the differences in findings. For instance, some associations were only in Pima Indians or Japanese subjects.[17,30,53] Finally, the variety in the prevalence of environmental risk factors might explain the diversity in the strength of the associations. For example, if there is only an effect of the genetic predisposition in the presence of another environmental factor for weight gain, it is likely that associations will be found preferentially in these populations in which

the environmental factor while in other population where the risk factor is rare, no associations will be found.

Thus, the ADRB2-Gly16Arg polymorphism was associated with weight gain in our population. However for several reasons, one should be careful with drawing conclusion from this. As we used a significance level of 0.05 and conducted many analyses, we cannot rule out chance as an explanation. Furthermore, the direction of the association among men is equivocal. As receptor function experiments have shown that the Gly16Arg polymorphism in the ADRB2 gene is associated with an altered receptor function one would expect a higher prevalence of the Gly/Gly genotype among the weight gainers compared to that of the non-weight gainers.[21,54] Our study is not the first one to show sex-specific associations for the ADRB2 gene. Hellström *et al* reported a higher prevalence of the Glu27 variant of the ADRB2 gene among female obese, compared to a decreased prevalence among male obese.[28] Meirhaeghe *et al* suggested that among women an altered pathway may be more efficiently compensated by a decrease in the alpha 2 adrenergic pathway compared to men.[26] Therefore, more refined association studies and experimental work on this polymorphism are needed.

From all genetic marker combinations, we found only four combinations that were associated with weight gain. However, given the number of statistical tests performed, it is likely that they are by chance. Before we draw conclusions from these analyses, these findings have to be confirmed in other studies. For this reason, we are currently increasing the sample size with the aim of confirming some of the associations and investigating the interactions between genes and between genes and lifestyle factors.

Since we only observed weak associations between genetic and behavioral factors with weight gain, the question remains why the majority of persons who gain weight do so. Our observation that not the food habits during breakfast, lunch and dinner are important (such as type of spread used and so on), but to some extent the use of alcohol and savory snacks deserves further study. In addition, leisure inactivity was shown to be predictive of weight gain in women. The association between lifestyle factors and weight gain requires further study.

In summary, none of the studied genetic markers are clearly associated with weight gain. Only variations in the ADRB2 gene and the LEPR gene may contribute to the susceptibility to weight gain, but replication studies are needed. Further research is necessary to establish the role of lifestyle factors, or interactions between genes or between genes and lifestyle factors on weight gain.

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